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LETTERS TO THE EDITOR

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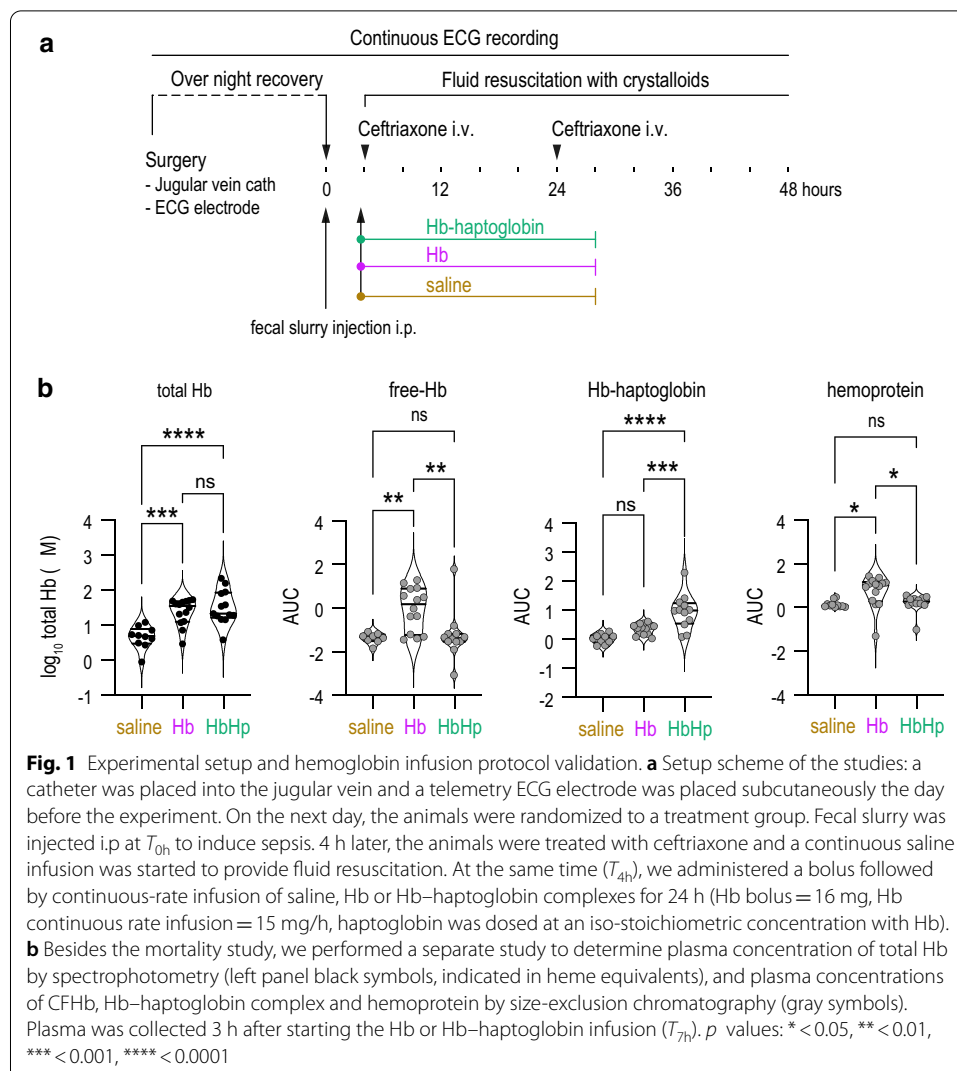
Haptoglobin treatment prevents cell-free hemoglobin exacerbated mortality in experimental rat sepsis

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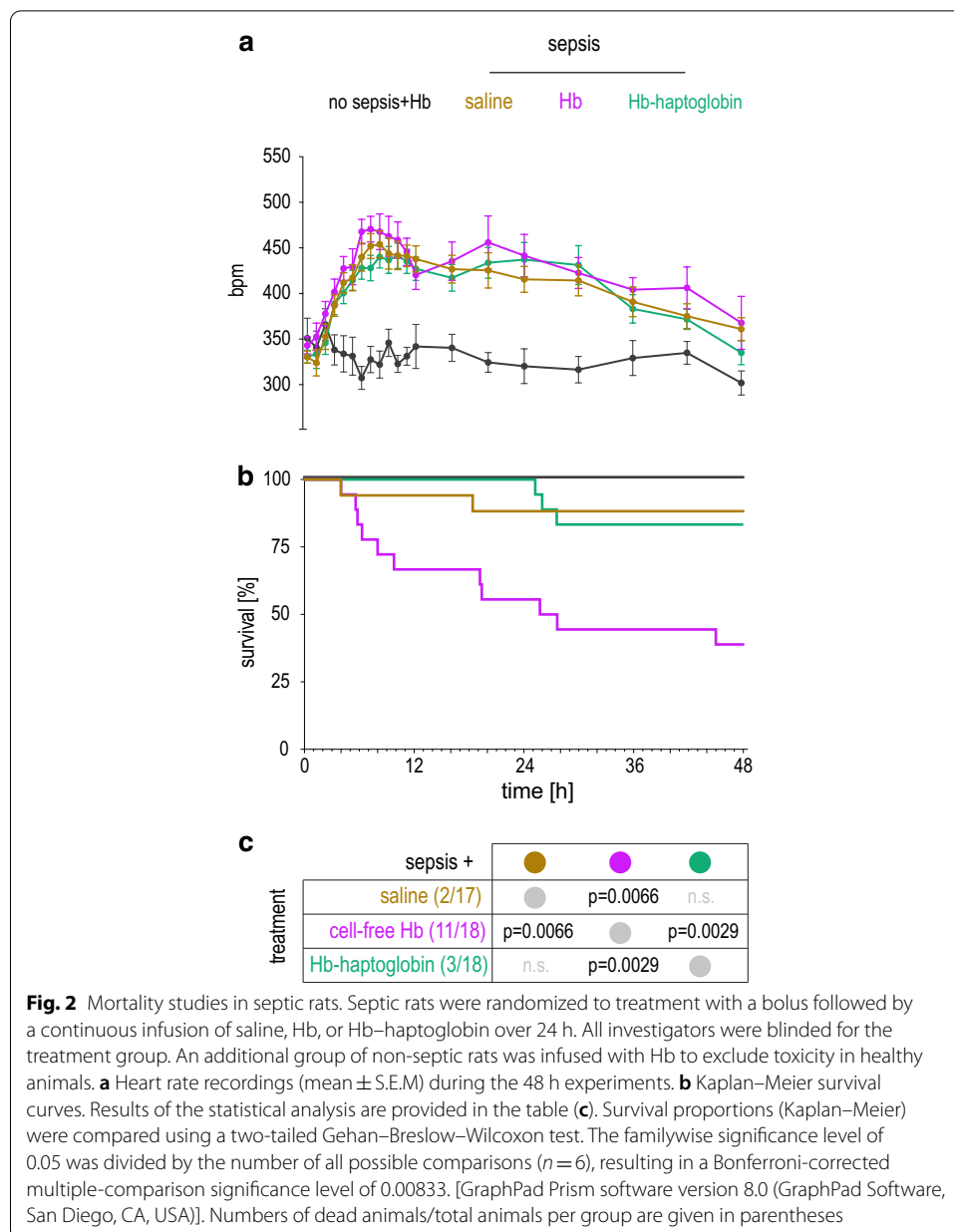
Sepsis is a dysregulated host response to infection leading to organ dysfunction, organ failure, and death. Multiple mechanisms promote hemolysis during sepsis, such as complement activation, disseminated intravascular coagulation, hemolytic pathogens, sepsis-induced erythrocyte dysfunction, blood transfusion, and medical procedures with extracorporeal circulation (e.g., renal replacement therapy) [1]. Clinical observations suggested that hemolysis with increased cell-free hemoglobin (CFHb) in plasma correlated with reduced survival in sepsis patients [2–4]. CFHb is a toxin, which may worsen sepsis pathophysiology by nitric oxide depletion, oxidative tissue injury, activation of coagulation and innate immune pathways, and as an iron source for pathogens [5]. The acute phase protein haptoglobin is the archetypical Hb scavenger in plasma and irreversibly neutralizes the toxicity of bound Hb [1].

Here, we performed a prospective, randomized, blinded animal study to provide direct experimental evidence that CFHb exacerbates sepsis mortality and test whether haptoglobin administration could revert this potentially detrimental adverse effect of hemolysis. For this, we used a fluid resuscitated fecal peritonitis model in awake rats that we have characterized in detail earlier (Fig. 1a) [6]. We first validated an Hb-administration protocol in 36 septic rats randomized to saline, CFHb, or Hb–haptoglobin infusion. Three hours after a bolus followed by continuous infusion, the mean total Hb concentrations in plasma were 5.4 μM (SD \pm 3.2 μM) in the saline group and 30.4 μM (SD \pm 17.3 μM) in the Hb group (Fig. 1b). These data confirmed that our infusion protocol resulted in plasma concentrations within the range of CFHb observed in patients with severe sepsis [2, 4]. Co-administration of human plasma-derived haptoglobin prevented Hb's renal clearance, resulting in higher concentrations than in the CFHb group (54.7 μM \pm 63.0 μM). We determined the fractions of CFHb, Hb–haptoglobin complexes, and heme-protein adducts by size-exclusion chromatography. CFHb and heme-protein adducts eluting in the albumin region remained suppressed when haptoglobin was administered concomitantly with CFHb. This confirms that Hb remains stabilized in the Hb–haptoglobin complex for



prolonged periods in circulation and that the complex efficiently prevents Hb degradation and heme release from CFHb [7, 8].

In the main study, we randomized 54 septic Wistar rats to treatment with saline, CFHb, or Hb–haptoglobin. One animal randomized to the saline group had to be excluded from the study, because the intravenous catheter was dislocated during the experiment. In addition, five non-septic animals were infused with CFHb to exclude acute Hb toxicity in healthy animals. After fecal slurry injection, tachycardia developed in all treatment groups consistent with a systemic inflammatory response (i.e., sepsis) (Fig. 2a). The exact timepoint of animal death was determined based on ECG telemetry recordings. The survival data provided evidence for a significantly higher mortality in the group of septic rats infused with CFHb compared to the septic animals infused with only saline (61% versus 12%; $p = 0.0066$). Co-administration of haptoglobin with CFHb improved mortality to 17%, which was not significantly different from the saline infusion group (12%) (Fig. 2b).



Previous reports demonstrated that blood transfusion-induced hemolysis caused excess mortality in a canine model of *S. aureus* pneumonia [5]. In the same model, administration of a haptoglobin concentrate improved shock, lung injury, and survival, suggesting that Hb-scavenging neutralized the adverse effects of CFHb [5]. With our model, we now provide direct evidence that purified Hb administered to reach clinically relevant plasma concentrations acts as a toxin during hemolysis, mimicking the adverse effect of intrinsic hemolysis. Our data collectively suggest that CFHb is a contributor to adverse sepsis outcomes and may provide a rationale for therapeutic haptoglobin supplementation as a strategy to improve clinical sepsis management.

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Authors' contributions

CAS designed the study, performed experiments, analyzed data, wrote the paper; VJ designed the study, performed experiments, analyzed data; TG analyzed plasma samples; DRS and AR designed the study; FV wrote the paper; DJS designed the study, analyzed data. All authors read and approved the final manuscript.

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Availability of data and materials

Original data are available upon reasonable request from the corresponding author.

Declarations**Ethical approval and consent to participate**

This animal study was approved by the Veterinary Office of the Kanton Zurich, Switzerland.

Consent for publication

Not applicable for this animal study.

Competing interests

The authors declare that they have no competing interests.

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